Final Report

Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats

(Study code : GT13-00016)

January 2014

the way to trust KCL Korea Conformity Laboratories

BioConvergence Technology Laboratory

Statement

Study code : GT13-00016

Title : Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats

This final report was written in Korean and translated into English.

This study has been performed in compliance with the principles of Good Laboratory Practices and test guidelines in following documents.

1. Standards of Good Laboratory Practice, National Institute of Environment Research (NIER)[Notice No. 2013-1 (revised 9th, Jan., 2013)]
2. Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER)[Notice No. 2013-2 (revised 9th, Jan., 2013)]
3. OECD Guidelines for the Testing of Chemical No. 402 'Acute Dermal Toxicity' (Adopted 24th Feb., 1987)

The stated object in study protocol was achieved and there were no significant deviations from the aforementioned regulations that affected the quality or integrity of the study. Therefore the justification of all data in this study was confirmed. The information of the test substance was written from the document that the sponsor provided.

Kyu-Sup Ahn — Jan., 02. 2014

Kyu-Sup Ahn
Study Director
BioConvergence Technology Laboratory

Date

Jin Kyu Lee — Jan, 02, 2014

Jin-Kyu Lee
Managing Director
BioConvergence Technology Laboratory

Date

QUALITY ASSURANCE STATEMENT

Study No. : GT13-00016

Title : Acute Dermal Toxicity Study of MWCNT in Spraque-Dawley Rats

This study was subject to audit by the independent Quality Assurance Unit of KCL as indicated below. The findings of each audit were reported to the study director and management as prescribed by Standard Operating Procedures.

The final report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the final report accurately reflect the raw data produced during the study.

Audit phases and dates reported to the responsible personnel were as indicated below and these were based upon the audit records.

Phase Inspected	Date	Reports to Study Director	Reports to Management
Study Plan	2013. 04. 12	2013. 04. 12	2013. 04. 12
Storage of Test substance and vehicle	2013. 04. 17	2013. 04. 17	2013. 04. 17
Animal receipt	2013. 04. 17	2013. 04. 17	2013. 04. 17
Preparation of test substance	2013. 04. 24	2013. 04. 24	2013. 04. 24
Animal care and Administration	2013. 04. 24	2013. 04. 24	2013. 04. 24
Clinical sign	2013. 05. 08	2013. 05. 08	2013. 05. 08
Necropsy	2013. 05. 08	2013. 05. 08	2013. 05. 08
Raw data	2013. 06. 10	2013. 06. 10	2013. 06. 10
Final Report	2013. 06. 10	2013. 06. 10	2013. 06. 10

QA director : Kuk, Won Kwen Ph.D. Date 2013. 06. 10
Auditor, Quality Assurance

* signed original

Study Personnel

Principal Investigator	Su-Chan Lee*	**Date**	06 June 2013
Formulation	Jae-Hyuck Sung*	**Date**	06 June 2013
Animal care	Min-Won Baek*	**Date**	06 June 2013
Necropsy & Pathology	Hye-Jin Kim*	**Date**	06 June 2013
Archiving	Hyo-Dong Kim*	**Date**	06 June 2013

* Signed original

Title	Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley
Objective of Study	This study is performed to assess the acute dermal toxicity and lethal dose 50 (LD_{50}) of MWCNT when the test substance is administered in single dose to rats.
Sponsor	Name : Bioconvergence Technology Laboratory Korea Conformity Laboratories Address : 7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea Tel. : 032-858-0011 Fax : 032-858-0020
Testing facility	Name : Bioconvergence Technology Laboratory Korea Conformity Laboratories Address : 7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea Tel. : 032-858-0011 Fax : 032-858-0020
Study Schedule	Animal acquisition : 17 April 2013 Administration : 24 April 2013 Necropsy : 08 May 2013 Submission of final report : 02 January 2014
Archiving of study data	1) Archiving period : least 5 years after the study termination 2) Data : Study protocol, test substance data, animal acquisition data, raw data, final report and GLP documents 3) Storage room (1) Archive : CD, relevant document

Contents

1. SUMMARY

This study has been performed to evaluate the lethal dose 50 (LD_{50}) and toxicity of the test substance MWCNT when it was administered in single dermal dose to Sprague-Dawley (SD) female rats. The limited test was conducted with the vehicle and 2,000 ㎎/㎏ dosing groups and during the study period, dead animals, clinical signs, body weight changes and gross findings at necropsy were examined.

1) No mortalities and unusual clinical signs were observed during the observation period in all animals.

2) In body weight, there were no body weight changes related with the test substance and no significant differences between vehicle control and dosing group.

3) At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

Under these conditions, the acute oral lethal dose 50 (LD_{50}) of the test substance MWCNT is considered greater than 2,000 ㎎/㎏ body weight in female Sprague-Dawley rats.

2. TEST SUBSTANCE AND VEHICLE

1) Test substance (Annex 1)

(1) Name : MWCNT (Kumho : K-Nanos-100P)

(2) CAS No. : -

(3) Lot No. : -

(4) Received date : 25 January 2013

(5) Received quantity : 666.89 g (including container weight)

(6) Molecular weight : -

(7) Appearance : powder

(8) Purity : >90%

(9) Solubility : -

(10) Stability : -

(11) Storage condition : -

(12) Handling

① Wear protection equipments including gloves, mask, glasses and clothes.

② Keep the test substance in seal container.

③ Keep the test substance in low humanity and good ventilated condition.

(13) Supplier : Kumho Petrochmical Co., Ltd.

2) Vehicles

(1) Vehicle 1

① Name : 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

② Lot No. : 078K5203

③ CAS No. : 63-89-8

④ Molecular weight : 734.04

⑤ Received date : 21 March 2012

⑥ Received quantity : 1 g

⑦ Appearance : White powder

⑧ Purity : ≥99%

⑨ Storage condition : In freezer

⑩ Manufacturer : Sigma-Aldrich, Inc.

(2) Vehicle 2

① Name : Dulbecco's phosphate buffered saline (DPBS)

② Lot No. : 031M8307

③ CAS No. : -

④ Received date : 08 May 2012

⑤ Received quantity : 480 g
⑥ Appearance : White solid
⑦ Storage condition : Refrigeration
⑧ Manufacturer : Sigma-Aldrich, Inc.

(3) Vehicle 3
① Name : D-(+)-Glucose
② Lot No. : 071M0145V
③ CAS No. : 50-99-7
④ Received date : 28 August 2012
⑤ Received quantity : 1 ㎏
⑥ Appearance : White powder
⑦ Storage condition : At room temperature
⑧ Manufacturer : Sigma-Aldrich, Inc.

(4) Vehicle 4
① Name : Bovine serum albumin
② Lot No. : 750462
③ CAS No. : -
④ Received date : 06 April 2009
⑤ Received quantity : 100 g
⑥ Appearance : Yellow powder
⑦ Storage condition : Refrigeration
⑧ Manufacturer : Gibco

3) Justification for vehicle choice

The test substance was not dispersed in ordinary vehicles. So the DPPC solution (5.5 mM D-(+)-glucose+0.6 mg/ml Bovine serum albumin+0.01 ㎎/㎏ DPPC in DPBS) was selected as vehicle base on the reference. (Jin Sik Kim et al, 2011, Evaluation of biocompatible dispersants for carbon nanotube toxicity tests, Arch Toxicol, 204:723) At the result of solubility test, the test substance was dispersed equally up to 1% concentration in DPPC solution.

4) Storage and Treatment

The test substance was kept in a storage room (108-2). There is no mention about the store condition of the test solution because it was prepared in the morning of the administration day.

5) Preparation of the test solution

The test substance was prepared respectively as much as the weight which corresponds to 2,000 ㎎/㎏ dose level according to a body weight of each animal on the administration day. And the vehicle, DPPC solution was also prepared in order to wet the test substance. The stability test was not performed because they were mixed just prior to the administration.

3. MATERIALS AND METHODS

1) Test animals

(1) Species and strains : Specific Pathogen Free(SPF) Sprague-Dawley(SD) rats

(2) Producer and Supplier

ORIENT BIO INC. (Address; 143-1, Sangdaewondong, Jungwon-gu, Seongnam-si, Gyeonggi-do, Korea)

(3) Reason for selection of the species

SD rats have been applied widely in general toxicity tests as a suitable experimental animal for toxicity testing. In addition, sufficient raw data has been accumulated and is available for interpretation and evaluation of study results.

(4) Date of acquisition : 17 April 2013

(5) Number of animals received : 11 males and 11 females, total 22 rats

(6) Age of animals received : 7 weeks

(7) Body weights on arrival

Male : 190.02～205.67 g

Female : 186.67～216.89 g

(8) Quarantine and acclimation

Animals were acclimated for 7 days. Only animals with the best appearance were selected for the test after observation during the acclimation period, Animals were accepted based on the certification provided by the supplier (Annex 2).

(9) Age at the initiation of the administration : 8 weeks

(10) Body weights at the administration

Male : 252.76～264.09 g

Female : 214.99～242.64 g

(11) Number of animals administered : 10 males and 10 females, total 20 rats

(12) Grouping

Animals were weighed one day before the test substance administration and

grouped to ensure a distribution of graded body weight.

(13) Identification of animals

Individual animals were identified by tail marking with an oily-ink felt-pen. Individual cages were distinguished by the individual card labeling. The record sheets provided at the entrance of the SPF animal room contained the study number, the study title, the duration of the SPF room use, the name of the study director and the names of study personnel.

(14) Disposal of remaining animals

They were treated by SOP of this testing facility

(15) Compliance with the guidelines of animal ethics

This study was approved by the IACUC of Korea conformity laboratory (approval number : IA13-00200).

2) Environmental and Housing Condition(Annex 3)

(1) Animal care room : Room 2 in the SPF animal facility area.

(2) Temperature and humidity : 23.0±0.9 ℃ and 50.1±6.7%RH

(3) Ventilation frequency : 10-15 air changes/hours

(4) Lighting cycle : 12 hours duration (lighting on at 8 a.m. and off at 8 p.m.)

(5) Lighting intensity : 285 Lux.

(6) Noise : 46.4 dB

(7) Concentration of ammonia : less than 5 ppm

(8) Housing

All animals were housed in wire mesh cages. (quarantine and acclimation period : 250W × 350L × 180H ㎜, administration and observation period : 150W × 350L × 180H ㎜) During the quarantine and acclimation period, not more than 3 animals were housed in a cage, whereas only an animal was housed in a cage during the administration and observation period. Cages were changed at grouping.

(9) Feeds and water

① Feeds

Radiation sterilized, solid laboratory animal feeds (Teklad Certified Irradiated Global 18 % Protein Rodent Diet, Harlan Co. Ltd., USA) were provided *ad libitum*. DooYeol Biotech Co., Ltd. supplied feeds.

② Water

Incheon, Korea municipal tap water purified by reverse osmosis filtering system was provided *ad libitum* using water bottles.

③ Certification

The feed certification which was provided from the supplier and the water certification from national certificated inspection organization were referred to examine contamination (Annex 4, 5).

3) Method

(1) Administration

① Route of administration and reason for the selection

The test substance was administrated to evaluate dermal toxicity.

② Method of administration

All animal's fur of dorsal skin was removed more than 20% of the area of body surface one day before the administration day. On the administration day, the test substance was laid on a gauze (5 × 5 cm) equally and then, the vehicle was added in order to wet the test substance. They were adhered to skin of a test animal using a non-irritation film (Tegaderm™ 1624W, 3M) and fixed by non-irritation tapes (Micropore™ 1530-1, 3M) and dressing bandages (Coban, 3M). In the vehicle control group, the vehicle was only administrated to test animals in the same manner as animals in the test group. After about 24 hours, they were removed from the application site and that site was washed using sterile distilled water.

③ Frequency and duration of administration

Single dose, in the morning of the administration day

④ Calculation of dosing weight

Individual dosing weight was adjusted based on fasted body weight measured right before the administration.

(2) Group Description

Group	Sex	Number of animals	Identification of animals	Dose volume (㎖/kg)	Dose level (㎎/kg)
G1	Male	5	G1-1～G1-5	-	0
	Female	5	G1-11～G1-15		
G2	Male	5	G2-6～G2-10	-	2,000
	Female	5	G2-16～G2-20		

G1 : Vehicle control group, G2 : Dosing group

(3) Determination of dose level

Dose levels were determined in accordance with 'Guideline for the Testing of Chemical Hazards', National Institute of Environment Research (NIER)[Notice No. 2013-2 (revised 9th, Jan., 2013)] and the OECD Guidelines for the

Testing of Chemical No. 402 'Acute Dermal Toxicity' (Adopted 24th Feb., 1987). If the study is performed as 2,000 ㎎/㎏ dose level used for the limited dose generally, the volume of an abovementioned dose level is too much that the test solution cannot be administrated. (volume : 200 ㎖/㎏, ∵ solubility : dispersion up to 1% in DPPC solution) Therefore, the test substance was applied directly without being dispersed in the vehicle.

(4) Observations and Examinations

① Clinical signs and mortalities

General clinical signs or mortalities of all treated animals were observed continuously during the first half-hour and the one hour from the administration time. After that, those animals were observed once hourly up to the first six hours on the administration day. From the next day, each animal was observed once every day up to 14 days after the administration.

② Body weight measurement

All individual animals were weighed before the administration and on 1, 7 and 14 days after the administration.

③ Necropsy and gross findings examination

On day 14 after the administration, all surviving animals were anesthetized with CO_2 gas, and terminated by exsanguination from the abdominal aorta and caudal vena cava. Complete post-mortem examinations were performed on all vital organs.

(6) Data analysis

Body weight changes of all animals in all groups were analysed through tables and figures that were applied to the mean value and the standard deviations. The differences between the vehicle control and the dosing groups were examined using the Independent Samples t-Test. SPSS for Windows version 12.0 software (SPSS, Chicago, IL, U.S.A.) was used for the analysis.

4. RESULTS

1) Mortalities

No mortalities were observed during the observation period in all animals.

2) Clinical signs

No unusual clinical signs were observed during the observation period in all animals.

3) Body weight changes

In each four animals of male vehicle control and 2,000 ㎎/㎏ dosing group (animal No. G1-1～G1-4, G2-6～G2-9) and all female animals, there were decreases in body weights at 1 day after administration compared with body weights before administration. Decreases of average weight were observed in animals of all groups during same period. Except that, there were normal body weight gains.

At the results of statistical analysis in body weight, there were no significant differences between vehicle control and dosing group.

4) Gross findings

At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

5. DISCUSSION AND CONCLUSION

This study has been performed to evaluate the lethal dose 50 (LD_{50}) and toxicity of the test substance MWCNT when it was administered in single dermal dose to Sprague-Dawley (SD) female rats. The limited test was conducted with the vehicle and 2,000 ㎎/㎏ dosing groups and during the study period, dead animals, clinical signs, body weight changes and gross findings at necropsy were examined.

No mortalities and unusual clinical signs were observed during the observation period in all animals.
In body weight, average body weights were temporarily decreased in animals of all groups at 1 day after administration. But these decreases were also observed in animals of the vehicle control group and it is considered that they were caused by bandaging stress. Therefore, it is considered to be not related with the test substance and there were no significant differences between vehicle control and dosing group.
At the end of the study, necropsy was conducted to all animals and no abnormal gross findings were observed.

Under these conditions, the acute oral lethal dose 50 (LD_{50}) of the test substance MWCNT is considered greater than 2,000 ㎎/㎏ body weight in female Sprague-Dawley rats.

6. REFERENCES

1) Standards of Good Laboratory Practice, National Institute of Environment Research (NIER)[Notice No. 2013-1 (revised 9th, Jan., 2013)]

2) Guideline for the Testing of Chemical Hazards, National Institute of Environment Research (NIER)[Notice No. 2013-2 (revised 9th, Jan., 2013)]

3) OECD Guidelines for the Testing of Chemical No. 402 'Acute Dermal Toxicity' (Adopted 24th Feb., 1987)

4) Jin Sik Kim et al, 2011, Evaluation of biocompatible dispersants for carbon nanotube toxicity tests, Arch Toxicol, 204:723

7. TABLES

Table 1. Mortalities and clinical signs of rats

SUMMARY OF MORTALITIES AND CLINICAL SIGNS			
STUDY : GT13-00016			SEX : MALE
		GROUP(㎎/㎏)	
		G1(0)	G2(2,000)
MORTALITIES	N	0/5	0/5
	%	0	0
CLINICAL SIGNS	Normal	5/5	5/5

			SEX : FEMALE
		GROUP(㎎/㎏)	
		G1(0)	G2(2,000)
MORTALITIES	N	0/5	0/5
	%	0	0
CLINICAL SIGNS	Normal	5/5	5/5

Number of animals with the signs / Number of animals examined

Table 2. Body weight changes of rats

SUMMARY OF BODY WEIGHT CHANGES(g)

STUDY : GT13-00016 SEX : MALE

Day	GROUP(㎎/㎏)							
	G1(0)				G2(2,000)			
0	258.97	±	4.15	(5)	259.40	±	4.03	(5)
1	255.73	±	6.38	(5)	254.31	±	6.03	(5)
7	312.80	±	8.79	(5)	313.27	±	12.94	(5)
14	365.13	±	14.51	(5)	358.79	±	20.11	(5)

SEX : FEMALE

Day	GROUP(㎎/㎏)							
	G1(0)				G2(2,000)			
0	228.36	±	8.66	(5)	232.22	±	9.90	(5)
1	224.62	±	8.61	(5)	223.84	±	14.22	(5)
7	248.05	±	8.06	(5)	252.69	±	18.11	(5)
14	265.36	±	10.25	(5)	270.40	±	27.30	(5)

Mean±S.D (Number of animals)

Table 3. Gross findings of rats

SUMMARY OF GROSS FINDINGS			
STUDY : GT13-00016			SEX : MALE
ORGAN	SIGN	GROUP(㎎/㎏)	
		G1(0)	G2(2,000)
All organs	Normal	5/5	5/5
			SEX : FEMALE
ORGAN	SIGN	GROUP(㎎/㎏)	
		G1(0)	G2(2,000)
All organs	Normal	5/5	5/5

Number of animals with the signs / Number of animals examinedr of animals examined

8. FIGURES

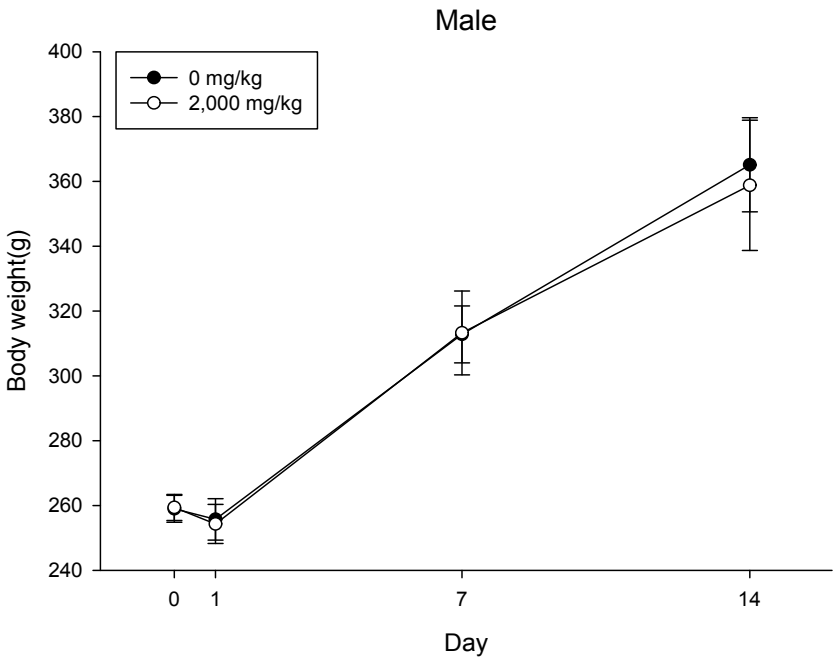


Figure 1. Body weight changes of male rat

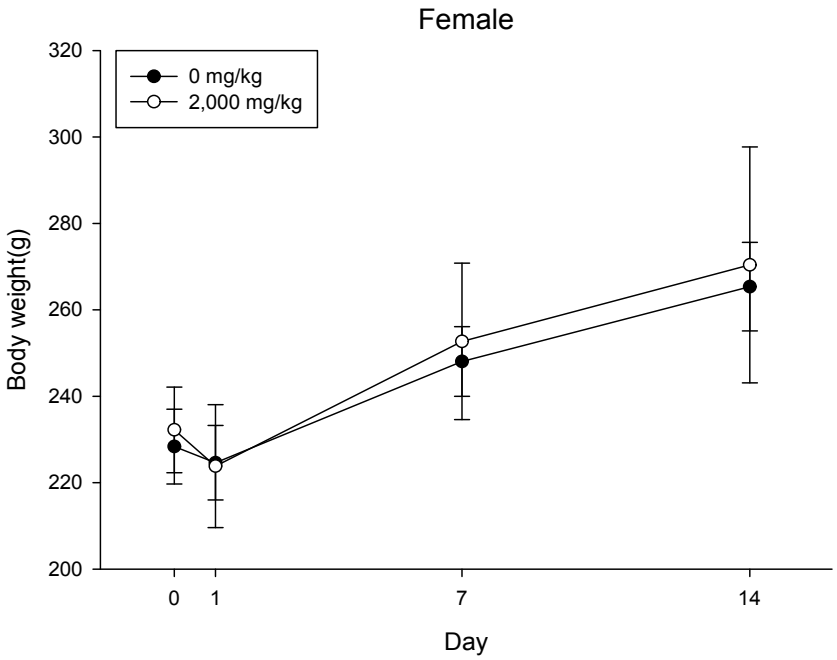


Figure 2. Body weight changes of female rat

9. APPENDICES

Appendix 1. Mortalities and clinical signs of rats

INDIVIDUAL DATA OF MORTALITIES AND CLINICAL SIGNS

STUDY : GT13-00016 SEX : MALE

GROUP (mg/kg)	ANIMAL ID	DATE DOSED	OBSERVATIONS	TIME OCCURRED
G1 (0)	G1-1	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-2	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-3	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-4	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-5	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
G2 (2,000)	G2-6	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-7	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-8	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-9	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-10	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14

SEX : FEMALE

GROUP (mg/kg)	ANIMAL ID	DATE DOSED	OBSERVATIONS	TIME OCCURRED
G1 (0)	G1-11	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-12	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-13	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-14	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G1-15	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
G2 (2,000)	G2-16	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-17	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-18	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-19	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14
	G2-20	24-Apr-2013	Normal Terminal sacrifice	Day 0 - 14 Day 14

Appendix 2. Body weight changes of rats

INDIVIDUAL DATA OF BODY WEIGHT CHANGES(g)

STUDY : GT13-00016 SEX : MALE

GROUP (mg/kg)	ANIMAL ID	Day 0	Day 1	Day 7	Day 14	Gain[a]
G1 (0)	G1-1	258.76	256.41	301.66	349.72	90.96
	G1-2	252.76	248.16	308.76	358.43	105.67
	G1-3	258.06	252.40	310.25	357.79	99.73
	G1-4	261.47	256.27	320.18	385.87	124.40
	G1-5	263.80	265.42	323.16	373.83	110.03
	Mean	258.97	255.73	312.80	365.13	106.16
	S.D.	4.15	6.38	8.79	14.51	12.45
G2 (2,000)	G2-6	257.89	251.30	307.73	348.00	90.11
	G2-7	253.77	246.34	293.66	331.17	77.40
	G2-8	258.89	254.42	316.97	359.30	100.41
	G2-9	264.09	257.13	321.59	374.53	110.44
	G2-10	262.34	262.38	326.38	380.94	118.60
	Mean	259.40	254.31	313.27	358.79	99.39
	S.D.	4.03	6.03	12.94	20.11	16.29

SEX : FEMALE

GROUP (mg/kg)	ANIMAL ID	Day 0	Day 1	Day 7	Day 14	Gain[a]
G1 (0)	G1-11	214.99	210.80	238.65	257.57	42.58
	G1-12	225.07	222.76	241.23	252.57	27.50
	G1-13	236.73	229.07	249.57	269.16	32.43
	G1-14	234.20	233.33	258.15	278.25	44.05
	G1-15	230.79	227.13	252.63	269.25	38.46
	Mean	228.36	224.62	248.05	265.36	37.00
	S.D.	8.66	8.61	8.06	10.25	6.97
G2 (2,000)	G2-16	220.84	212.48	230.83	240.10	19.26
	G2-17	222.87	206.55	243.68	258.90	36.03
	G2-18	234.74	227.01	256.29	286.83	52.09
	G2-19	240.00	231.73	252.78	257.21	17.21
	G2-20	242.64	241.41	279.89	308.97	66.33
	Mean	232.22	223.84	252.69	270.40	38.18
	S.D.	9.90	14.22	18.11	27.30	21.14

a : Body weight gains between day 0 and day 14

Appendix 3. Gross findings of rats

INDIVIDUAL DATA OF GROSS FINDINGS

STUDY : GT13-00016 SEX : MALE

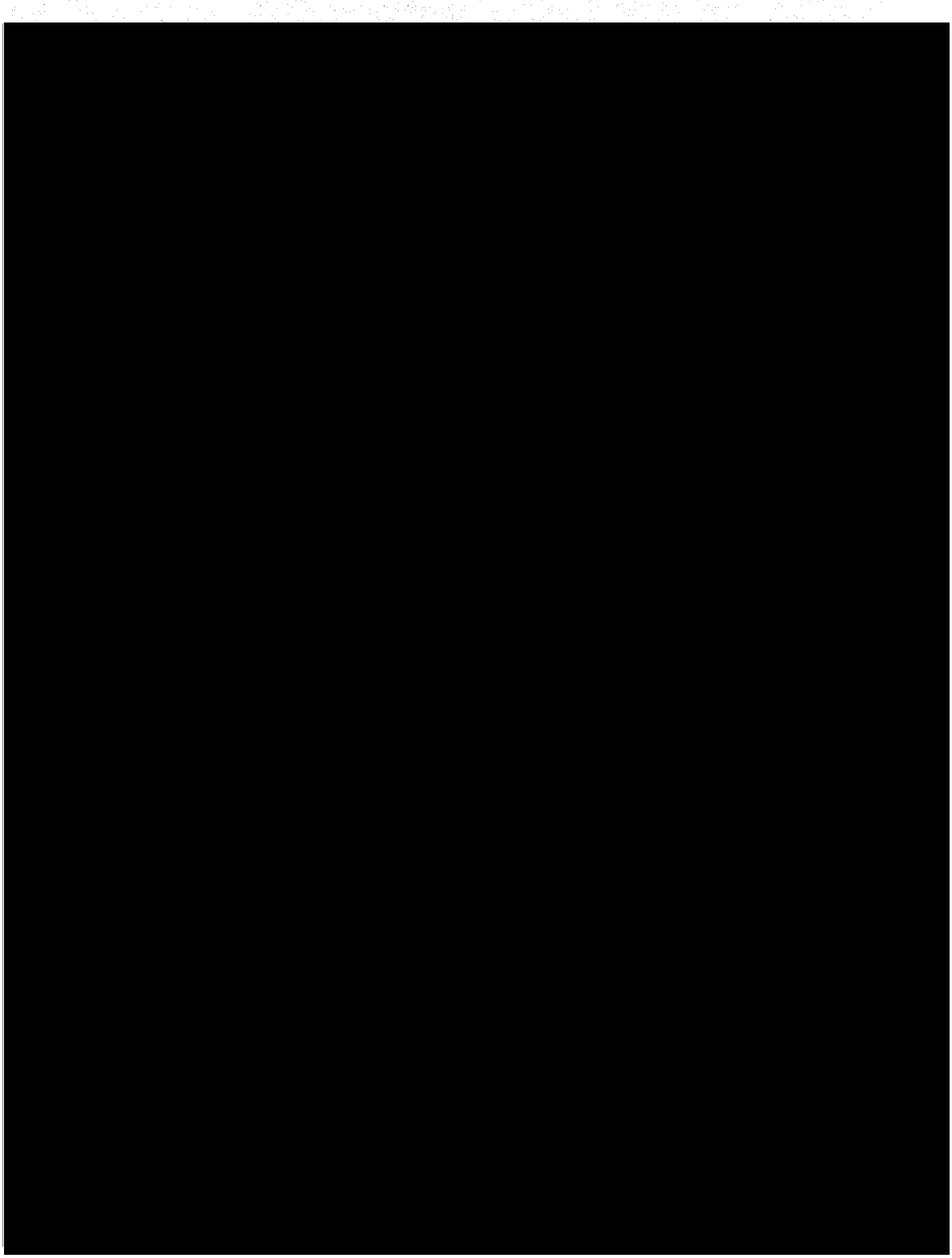
GROUP (㎎/㎏)	ANIMAL ID	FATE (DAY)	ORGAN	OBSERVATIONS
G1 (0)	G1-1	Terminal sacrifice (14)		No organ with gross findings
	G1-2	Terminal sacrifice (14)		No organ with gross findings
	G1-3	Terminal sacrifice (14)		No organ with gross findings
	G1-4	Terminal sacrifice (14)		No organ with gross findings
	G1-5	Terminal sacrifice (14)		No organ with gross findings
G2 (2,000)	G2-6	Terminal sacrifice (14)		No organ with gross findings
	G2-7	Terminal sacrifice (14)		No organ with gross findings
	G2-8	Terminal sacrifice (14)		No organ with gross findings
	G2-9	Terminal sacrifice (14)		No organ with gross findings
	G2-10	Terminal sacrifice (14)		No organ with gross findings

SEX : FEMALE

GROUP (㎎/㎏)	ANIMAL ID	FATE (DAY)	ORGAN	OBSERVATIONS
G1 (0)	G1-11	Terminal sacrifice (14)		No organ with gross findings
	G1-12	Terminal sacrifice (14)		No organ with gross findings
	G1-13	Terminal sacrifice (14)		No organ with gross findings
	G1-14	Terminal sacrifice (14)		No organ with gross findings
	G1-15	Terminal sacrifice (14)		No organ with gross findings
G2 (2,000)	G2-16	Terminal sacrifice (14)		No organ with gross findings
	G2-17	Terminal sacrifice (14)		No organ with gross findings
	G2-18	Terminal sacrifice (14)		No organ with gross findings
	G2-19	Terminal sacrifice (14)		No organ with gross findings
	G2-20	Terminal sacrifice (14)		No organ with gross findings

10.ANNEXES

Annex 1. Test substance chemical data sheet



Annex 2. Animal certification

Rat VAF Report

CR Rodent Production

Location: Orient Bio Inc. KP800 VAF Rat

Sponsor: Orient Bio Inc.

Colony: Crl:CD(SD) Colony # 28804

Reported: Monday, March 4, 2013 at 1:22

Summary Item	Primary Assay	Most Recent		Past 18 Months
		Year-Week	Positive / Tested	Positive / Tested
Virology				
SEND ae	MFIA	2013-05	0 / 8	0 / 48
PVM ae	MFIA	2013-05	0 / 8	0 / 48
SDAV ad	MFIA	2013-05	0 / 8	0 / 48
KRV ad	MFIA	2013-05	0 / 8	0 / 48
H1 ad	MFIA	2013-05	0 / 8	0 / 48
RPV ad	MFIA	2013-05	0 / 8	0 / 48
RMV ad	MFIA	2013-05	0 / 8	0 / 48
REO ae	MFIA	2013-05	0 / 8	0 / 48
RTV ad	MFIA	2013-05	0 / 8	0 / 48
LCMV ae	MFIA	2013-05	0 / 8	0 / 48
HANT ae	MFIA	2013-05	0 / 8	0 / 48
MAV ae	MFIA	2013-05	0 / 8	0 / 48
Microbiology				
B. bronchiseptica be	Culture	2013-05	0 / 8	0 / 48
CAR Bacillus ae	MFIA/PCR	2013-05	0 / 8	0 / 48
C. kutscheri ae	Culture	2013-05	0 / 8	0 / 48
H. bilis be	PCR	2013-05	0 / 8	0 / 48
H. hepaticus ae	PCR	2013-05	0 / 8	0 / 48
Helicobacter sp. be	PCR	2013-05	0 / 8	0 / 48
K. oxytoca ce	Culture	2013-05	0 / 8	0 / 48
K. pneumoniae ce	Culture	2013-05	0 / 8	0 / 48
M. pulmonis ae	MFIA	2013-05	0 / 8	0 / 48
P. multocida ce	Culture	2013-05	0 / 8	0 / 48
P. pneumotropica ce	Culture	2013-05	0 / 8	0 / 48
P. aeruginosa ce	Culture	2013-05	0 / 8	0 / 48
Salmonella spp. ae	Culture	2013-05	0 / 8	0 / 48
S. moniliformis af	PCR	2013-05	0 / 8	0 / 32
Strep. pneumoniae be	Culture	2013-05	0 / 8	0 / 48
Pneumocystis ("RRV") bd	MFIA	2013-05	0 / 8	0 / 48
Tyzzer's Disease ag	Exam	2013-05	0 / 8	0 / 48
Pathology				
Gross Exam ei	Exam, Histopathology	2013-05	0 / 8	0 / 48
Parasitology				
Ectoparasites ae	Exam	2013-05	0 / 8	0 / 48
Helminths ae	Exam	2013-05	0 / 8	0 / 48
Giardia sp. be	Exam	2013-05	0 / 8	0 / 28
Spironucleus sp. be	Exam	2013-05	0 / 8	0 / 28
Other Protozoa ce	Exam	2013-05	0 / 8	0 / 28
E. cuniculi ae	MFIA	2013-05	0 / 8	0 / 48

COLONY POLICY FOR POSITIVE RESULT: a = immediate termination; b = planned future recycle of the colony; c = no action.
TESTING SCHEDULE: d = screened every four weeks; e = screened quarterly; f = screened annually; g = screened quarterly by clinical exam.
1 = results do not include incidental or strain related findings; significant findings would result in immediate termination of the colony.

Charles River RADS-ILIMS Report **Page 1 of 1**

Annex 3. Environmental certification of animal care room

Certification of Environment for animal breeding room

Study No.	GT13-00016
Title	Acute Dermal Toxicity Study of MWCNT in Sprague-Dawley Rats
SPF Room No.	SPF #2 Animal Room
Period of animal Breeding	2013 - 04 - 17 ~ 2013 -05 - 08

Breeding environment condition

Section	Range of SOP	Survey value	Remark
Temperature	22±3 ℃	23.0±0.9 ℃	
Humidity	50±20 %RH	50.1±6.7 %RH	
Luminous intensity	150~300 Lux	285 Lux	
Noise	60 dB less than	46.4 dB	
Ammonia	15 ppm less than	5 ppm less than	

It is authenticated that there is no change of environment which digresses from the above established value for more than 2 hours during the test period.

Facility management director Dong-Seok Beck (sign)

2013-12-31

Annex 4. Laboratory animal diet certification

Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-120212MA
Date of Manufacture 12/02/12
Report Date 12/18/12

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Kurt Schafer
Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2012.12.21 07:18:28
-06'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.40
Fat	6.14
Fiber	3.32
Moisture	12.00
Ash	5.51
Calcium	0.98
Phosphorus	0.68

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.17	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.24	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Annex 4. Laboratory animal diet certification (continued)

Laboratory Diet Certification Report

Teklad Certified Irradiated Global 18% Protein Rodent Diet

2918C

Lot Number 2918C-030413MA
Date of Manufacture 03/04/13
Report Date 03/19/13

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

Kurt Schafer
Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2013.03.20 09:52:32 -05'00'

Proximate Analysis

Analysis	Result (%)
Protein	18.20
Fat	6.17
Fiber	3.82
Moisture	10.50
Ash	5.66
Calcium	1.01
Phosphorus	0.77

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	0.12	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.34	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

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Annex 5. Certification of water analysis

TEST REPORT

Reissuance (R1)
Date : 2013.4.11

1. No : PC13-00284
2. Client
   - o Name : Korea Conformity Laboratories(Incheon)
   - o Address : #7-44, Songdo-dong, Yeonsu-gu, Incheon, Korea
   - o Date of Receipt : Mar. 14, 2013
   - o Date of Issued : Apr. 17, 2013
3. Use of Report : Submission
4. Test Sample : Drinking Water (Animal room)
5. Method :
   (1) Notification No.2012-143 of the Ministry of Environment.

Affirmation	Tested By Name : Hyoung jun Seok Seok.	Technical Manager Name : Sang Cheul Lee S.C. Lee

Our report apply only to the standards or procedures identified and to the sample(s) tested unless otherwise specified.
The test results are not indicative of representative of the qualities of the qualities of the lot from which the sample was taken or of apparently identical or similar products.

Korea Conformity Laboratories President Song Jae Bin Jae Bin Song

Address : 704-932 277-5, Jukjeon-Dong, Dalseo-Gu, Daegu,704-932,Korea 82-53-557-6681
Result Inquiry : Environmental Testing Center 82-2-2102-2598

- Page 1 of 3 -

QP-20-01-07(2)

Annex 5. Certification of water analysis (continued)

TEST REPORT

No : PC13-00284

6. Test Results

1) Drinking Water (Animal room)

Test Item(s)	Unit	Limitation(s)	LOQ	Test method used	Test Result(s)
Total colony counts	CFU/mL	Less than 100	0	(1)	0
Total coliforms	-/(100mL)	Not detected	-	(1)	Not detected
E-Coli	-/(100mL)	Not detected	-	(1)	Not detected
Lead	mg/L	Less than 0.01	0.005	(1)	Not detected
Arsenic	mg/L	Less than 0.01	0.005	(1)	Not detected
Selenium	mg/L	Less than 0.01	0.005	(1)	Not detected
Cadmium	mg/L	Less than 0.005	0.002	(1)	Not detected
Bolon	mg/L	Less than 1.0	0.01	(1)	Not detected
Copper	mg/L	Less than 1.0	0.008	(1)	Not detected
Zinc	mg/L	Less than 3.0	0.002	(1)	0.003
Iron	mg/L	Less than 0.3	0.05	(1)	Not detected
Manganese	mg/L	Less than 0.3	0.005	(1)	Not detected
Aluminium	mg/L	Less than 0.2	0.02	(1)	Not detected
Mercury	mg/L	Less than 0.001	0.001	(1)	Not detected
Fluoride	mg/L	Less than 1.5	0.15	(1)	Not detected
Nitrate nitrogen	mg/L	Less than 10	0.1	(1)	0.2
Chloride	mg/L	Less than 250	0.4	(1)	0.6
Sulfate	mg/L	Less than 200	2	(1)	Not detected
Diazinon	mg/L	Less than 0.02	0.000 5	(1)	Not detected
Parathion	mg/L	Less than 0.06	0.000 5	(1)	Not detected
Fenitrotion	mg/L	Less than 0.04	0.000 5	(1)	Not detected
Dichloromethane	mg/L	Less than 0.02	0.002	(1)	Not detected
1,1,1-Trichloroethane	mg/L	Less than 0.1	0.001	(1)	Not detected
Benzene	mg/L	Less than 0.01	0.001	(1)	Not detected
Toluene	mg/L	Less than 0.7	0.001	(1)	Not detected
Ethylbenzene	mg/L	Less than 0.3	0.001	(1)	Not detected
Xylene	mg/L	Less than 0.5	0.001	(1)	Not detected
1,1-Dichloroethylene	mg/L	Less than 0.03	0.001	(1)	Not detected
Tetrachlorocarbon	mg/L	Less than 0.002	0.001	(1)	Not detected
Tetrachloroethylene	mg/L	Less than 0.01	0.001	(1)	Not detected

Annex 5. Certification of water analysis (continued)

TEST REPORT

No : PC13-00284

6. Test Results

1) Drinking Water (Animal room)

Test Item(s)	Unit	Limitation(s)	LOQ	Test method used	Test Result(s)
Trichloroethylene	mg/L	Less than 0.03	0.001	(1)	Not detected
1,2-Dibromo-3-Chloropropane	mg/L	Less than 0.003	0.001	(1)	Not detected
Carbaryl	mg/L	Less than 0.07	0.005	(1)	Not detected
Chromium	mg/L	Less than 0.05	0.03	(1)	Not detected
Ammonium Nitrogen	mg/L	Less than 0.5	0.01	(1)	Not detected
Phenol	mg/L	Less than 0.005	0.005	(1)	Not detected
Alkyl Benzene Sulfate	mg/L	Less than 0.5	0.1	(1)	Not detected
Cyanide	mg/L	Less than 0.01	0.01	(1)	Not detected
pH	-	5.8 ~ 8.5	-	(1)	6.2
Turbidity	NTU	Less than 1	0.02	(1)	0.11
Color	degree	Less than 5	1	(1)	Not detected
Taste	-	Free	-	(1)	Pass
Odor	-	Free	-	(1)	Pass
Hardness	mg/L	Less than 300	1	(1)	Not detected
Consumption of $KMnO_4$	mg/L	Less than 10	0.3	(1)	0.6
Total solids	mg/L	Less than 500	2	(1)	Not detected

—— End of Report ——

- Page 3 of 3 -

QP-20-01-08(2)

Annex 6. KCL GLP certificate

지정번호 (Certification No.) 제 2008-4 호		화학물질 유해성 시험기관 지정서 GLP Certificate
①	시험기관	한국생활환경시험연구원 안전성평가본부
	Test Facility Name	Korea Environment and Merchandise Testing Institute Bio-Safety Evaluation Headquarters
②	소 재 지	인천광역시 연수구 송도동 7-44
	Address	7-44, Songdo-Dong, Yeonsu-Gu, Incheon, 406-840, Korea
③	대 표 자	김 창 로
	President	Chang-Ro Kim
④	운영책임자	유 일 재
	Test Facility Management	Il-Je Yu
⑤	시험의 범위	- 급성경구독성시험, 유전독성시험(복귀돌연변이시험, 염색체이상시험, 소핵시험). (유효기간 : 2006년 3월 31일부터). 끝. - 급성피부자극성 및 부식성시험, 급성안자극성 및 부식성시험, 급성흡입독성시험. (유효기간 : 2007년 4월 17일부터). 끝. - 아급성독성시험, 피부감작성시험. (유효기간 : 2008년 8월 25일부터). 끝.
	Test Scope	- Acute oral toxicity, Genetic Toxicity(Ames test, Chromosome abberation test, Micronucleus test) (Validation : since Mar. 31, 2006). - Acute dermal irritation/corrosion, Acute eye irritation/ corrosion, Acute inhalation toxicity (Validation : since Apr. 17, 2007). - Subchronic toxicity, Skin sensitization (Validation : since Aug. 25, 2008).

「유해화학물질관리법」 제14조, 같은 법 시행령 제12조 및 같은 법 시행규칙 제10조제2항에 따라 화학물질 유해성 시험기관(GLP시험기관)으로 지정합니다.

It is hereby certified that the test facility was inspected by the national compliance monitoring authority regarding compliance with the Principles of Good Laboratory Practice.

Issue date 2008년(year) 8월(month) 25일(date)

국립환경과학원장 인

President, National Institute of Environmental Research

Annex 6. KCL GLP certificate (continued)

(뒤 쪽)-1

<변경사항>

일자	내용	확인
2009. 5. 20	운영책임자 변경 : 유 일 재 (Il-Je Yu) 에서 송 경 석 (Kyung-Seuk Song) 으로 변경	
2009. 11. 16 (추가)	시험의 범위 : 급성경피독성시험, 어류급성독성시험 (유효기간 : 2009년 11월 16일 부터) 끝.	
〃 (연장)	Test Scope : Acute dermal toxicity, Fish: acute toxicity (Validation : since Nov. 16, 2009).	
2010. 8. 2	대표자 변경 : 김 창 로 (Chang-ro Kim) 에서 오 태 식 (Taeshik Oh) 로 변경	G L P 확 인
2010. 8. 2	기관명 변경 : "한국산업융합연구원 생물융합기술센터 바이오융합본부"로 변경 *영문명 (Bioconvergence Technology Division, Korea Conformity Laboratories)	G L P 확 인
2011. 9. 9	운영책임자 변경 : 송 경 석 (Kyung-Seuk Song) 에서 이 진 규 (Jin Kyu Lee) 으로 변경	G L P 확 인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인
2010. 12.	정기적부평가 결과, GLP기준을 준수하고 있음 (GLP Compliance)	G L P 확 인

Annex 6. KCL GLP certificate (continued)

화학물질유해성시험기관 지정서
제2008-4호

(뒤 쪽)-2

<변경사항>

일자	내용	확인
2011. 9. 9	기관명변경: "한국건설생활환경시험연구원 바이오융합단"으로 변경 (Bioconvergence Technology Department, Korea Conformity Laboratories)	G L P 확 인
2011. 11. 3	대표자 변경 : 오태식 (Taeshik Oh)에서 송 재빈 (Jae Bin Song)으로 변경	G L P 확 인

<처분사항>

일자	내용	확인

<참고사항>

일자	내용	확인

Annex 7. Quality assurance statement-Original

신 뢰 성 보 증 확 인 서

시험번호 : GT13-00016

시 험 명 : Sprague-Dawley 랫드를 이용한 MWCNT의 급성경피독성시험

이 보고서에 기술된 시험을 독립적으로 아래와 같이 시험과정 단계별로 점검하였으며 각 점검결과를 표준작업지침서에 따라 시험책임자와 운영책임자에게 통보 및 보고하였다.

본 시험은 국립환경과학원 고시 제2013-1호(2013년 01월 09일) '화학물질유해성시험연구기관 지정 및 관리기준', 국립환경과학원 고시 제2013-2호(2013년 01월 09일) '화학물질유해성시험방법' 및 OECD Guidelines for the Testing of Chemical No. 402 'Acute Dermal Toxicity'(Adopted 24th Feb., 1987)에 따라 수행되었으며, 보고서 작성 방법 및 결과의 기술이 시험 실시과정에서 발생한 시험기초자료를 바탕으로 정확히 반영되었음을 확인하였다.

점 검 내 용	실 시 일	시험책임자에게 통보일	운영책임자에게 보고일
시험계획서 점검	2013. 04. 12	2013. 04. 12	2013. 04. 12
시험물질 및 대조물질	2013. 04. 17	2013. 04. 17	2013. 04. 17
동물입수	2013. 04. 17	2013. 04. 17	2013. 04. 17
시험물질조제	2013. 04. 24	2013. 04. 24	2013. 04. 24
동물사육 및 투여	2013. 04. 24	2013. 04. 24	2013. 04. 24
증상관찰 및 측정	2013. 05. 08	2013. 05. 08	2013. 05. 08
부검	2013. 05. 08	2013. 05. 08	2013. 05. 08
시험기초자료	2013. 06. 10	2013. 06. 10	2013. 06. 10
최종보고서 점검	2013. 06. 10	2013. 06. 10	2013. 06. 10

KOREAN SOCIETY OF QUALITY ASSURANCE KSQA Won-Kwen Kuk 2012-11 CERTIFIED QA PROFESSIONAL IN GLP

한국건설생활환경시험연구원 바이오융합연구소
신뢰성보증책임자 국원근

2013 년 06 월 10 일

Annex 8. Study personnel-Original

시험관계자 서명

주 시험담당자	이 수 찬 주 시험담당자	날짜	2013. 06. 10
시험물질 조제	성 재 혁 시험물질 조제분석 책임자	날짜	2013. 06. 10.
동물관리	배 민 원 동물관리 책임자	날짜	2013. 06. 10
부검 및 병리	김 혜 진 병리 책임자	날짜	2013. 06. 10.
자료보관	김 효 동 자료보관 책임자	날짜	2013. 06. 10